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SCANNING ELECTRON MICROSCOPY OF THE EGGS OF *AEDES VEXANS* AND *AEDES INFIRMATUS* (DIPTERA: CULICIDAE)

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Abstract.—Descriptions based on scanning electron micrographs are given of the eggs of *Aedes* (*Aedimorphus*) *vexans* (Meigen) and *Ae.* (*Ochlerotatus*) *infirmatus* Dyar and Knab. The intact surface detail of the eggs is shown, including the dorsal, lateral and ventral surfaces, and the posterior pole, anterior pole and micropyle. This is in contrast to earlier descriptions which were incomplete and which were based on eggs from which the outer chorion was removed.

Key Words: Insecta, mosquito, egg, fine structure, chorionic sculpturing, scanning electron microscopy

The eggs of *Aedes* (*Aedimorphus*) *vexans* (Meigen) and *Ae.* (*Ochlerotatus*) *infirmatus* Dyar and Knab were first described, respectively, by Horsfall and Craig (1956) and Craig and Horsfall (1960). The descriptions were augmented by phase contrast photomicrographs of the chorionic sculpturing after preparative methods (Craig 1955) that removed the outer chorion. In the case of *Ae. vexans,* material prepared in the same way was examined also by Myers (1967) and Kalpage and Brust (1968) to provide additional illustrations based on phase contrast microscopy. As the scanning electron microscope came into more general use, Horsfall et al. (1970) re-examined the egg of *Ae. vexans* and other species and published a number of electron micrographs showing several variants, including details of the chorionic sculpturing and micropyle. Again, however, the outer chorion was removed prior to examination, so that the micrographs do not show the structure of the intact egg. None of these earlier illustrations, either of *Ae. vexans, Ae. infirmatus,* or other species examined, show the intact outer chorion and real appearance of the eggs.

Despite the fact that structures of the outer chorion may not appear taxonomically useful under light microscopy (Myers 1967), they incorporate a potential for interspecific variation not found in the inner chorionic sculpturing, the simple reticulate outline created by the chorionic cell boundaries. Scanning electron microscopy, a technique now readily available to most workers, was used in this paper to provide more complete and quantitative descriptions of the intact eggs of *Ae. vexans* and *Ae. infirmatus,* including details of the anterior pole and micropyle, the posterior pole, and differences between the dorsal and ventral surfaces of the egg.

MATERIALS AND METHODS

Females of *Ae. vexans* were collected by aspiration in citrus groves within 8 km of the Florida Medical Entomology Laboratory, while *Ae. infirmatus* females were collected on the laboratory grounds. About 15

females of each species were allowed to take blood to repletion from the author's arm and were then enclosed individually in 2.5 × 4.0 cm cylindrical containers placed on damp cheese-cloth several layers thick. In a few days most females had laid some eggs, which were washed carefully into a single small dish with filtered distilled water. The eggs were then thoroughly mixed and pipetted onto small (<15 mm) circles of filter paper. The specimens were kept covered and allowed to embryonate fully, and were then dried first in air, then in a desiccator over calcium chloride for 24 h. The filter paper circles were fixed to stubs with silver paint and, 24 h later, coated with gold. Specimens were examined in a Hitachi S-510 scanning electron microscope.

The terminology follows Harbach and Knight (1980), except for the terms anterior ring and outer chorionic cell field, which are defined by Linley (1989).

Results

Aedes (*Aedimorphus*) *vexans* (Figs. 1–3)

Size: dimensions as in Table 1.

Color: dark bronze.

Overall appearance: shape variable, curvature of ventral surface greater than dorsal, greatest diameter somewhat anterior to middle, anterior taper more pronounced, posterior more gradual (Fig. 1). Outer chorionic cells uniformly elongate longitudinally (in long axis of egg), each more or less completely filled by longitudinally aligned outer chorionic tubercles. Tubercles occasionally in a single row, but usually in two rows, at least at widest part of cell. Micropylar collar indistinct.

Chorion, dorsal, lateral and ventral surfaces: all surfaces very similar (Fig. 1). Outer chorionic cells longitudinally elongate, 20–42 μm long, 8–12 μm wide (2.5–5 times as long as wide), irregularly polygonal with boundaries clearly defined but not very straight (Figs. 1, 2a, b). Cell fields 17–39 μm

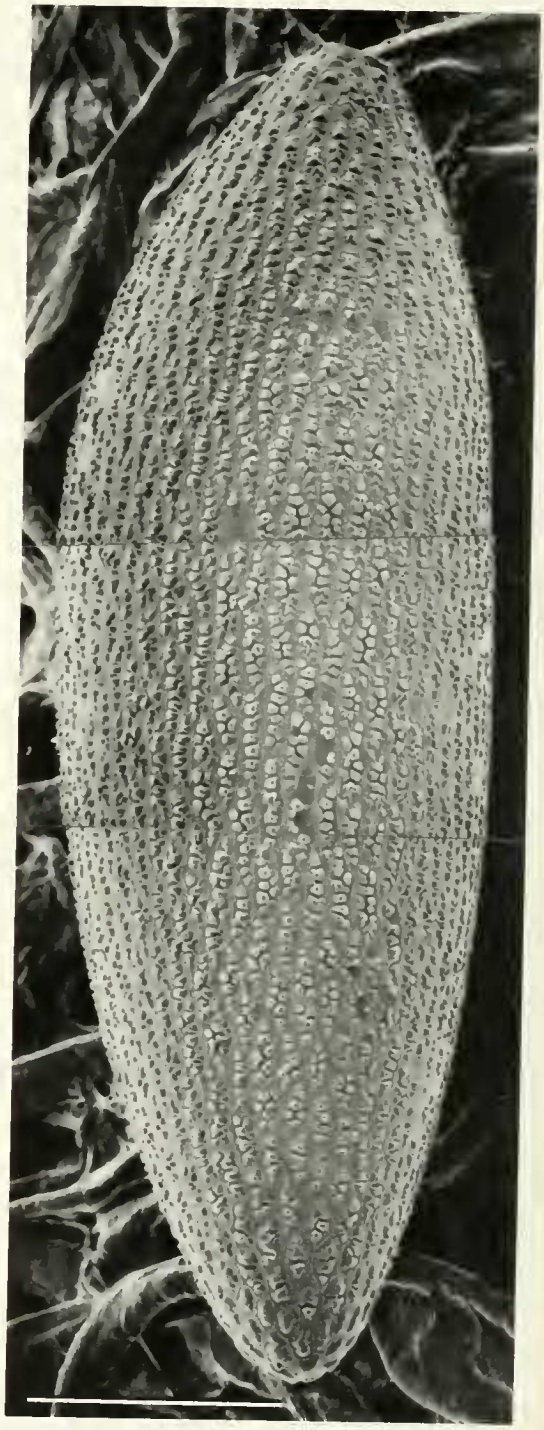


Fig. 1. *Ae. vexans.* Entire egg lateral view; dorsal surface at right, anterior end at top. Scale = 100 μm.

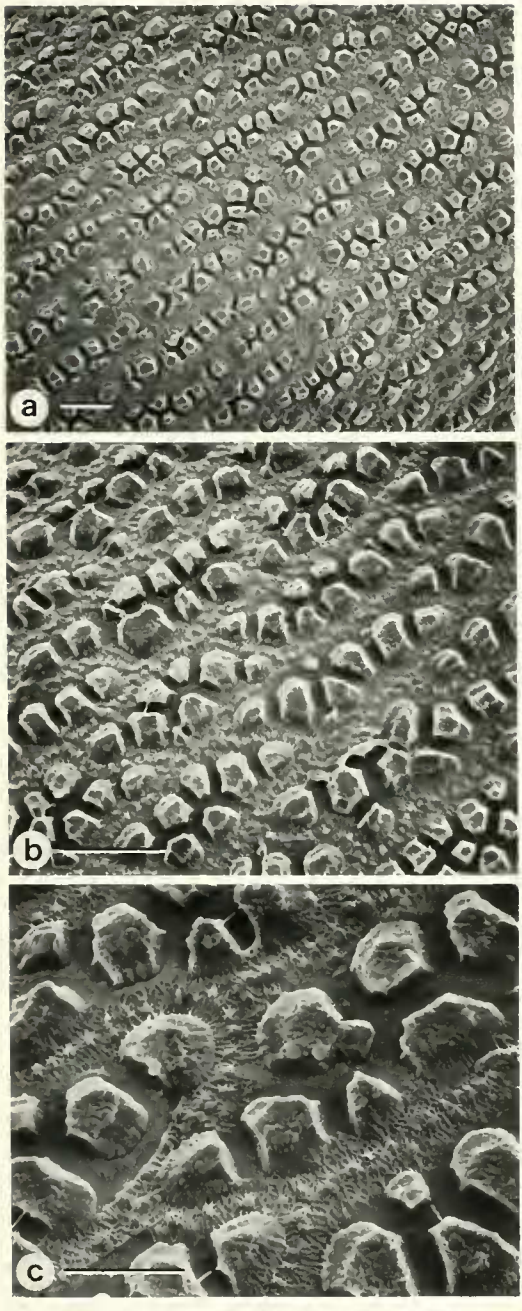


Fig. 2. *Ae. vexans.* (a) Outer chorionic cells, ventral surface, middle of egg; (b) detail of outer chorionic cells and tubercles; (c) detail of individual outer chorionic tubercles and outer chorionic reticulum. Scale = 10 μm (a, b), = 5 μm (c).

long, 7–10 μm wide, with smooth floors (Fig. 2c). Outer chorionic tubercles 6–15 in number, fewer per cell on dorsal surface than on ventral (lateral surface not counted), but not significantly so (Table 2). Tubercles arranged longitudinally in a single row, or more frequently a double row at widest parts of cell (Fig. 2a, b). Outer edges of tubercles almost always touching outer chorionic reticulum, gaps separating tubercles strikingly uniform, ca. 1.5 μm (Fig. 2a, b).

Shape of tubercles irregular, roughly polygonal, shapes of edges tending to match those of adjacent tubercles (Fig. 2b), largest tubercles ca. 5.5 μm in longest dimension, smallest ca 2.4 μm, but very small tubercles uncommon. In detailed structure, each tubercle consists of a base, often with slightly concave inner edges with sloped, tapered walls rising to a smaller, flat top ornamented with poorly defined bumps and fissures (Fig. 2b, c). Outer edges of tubercle bases usually rounded (Fig. 2c). Outer chorionic reticulum low, width 1.2–3.5 μm, consisting of a very fine reticulate meshwork with central line of small, bead-like protuberances (Fig. 2b, c), more or less evenly spaced (1.0–3.2 μm). Meshwork usually touching and continuing some distance up sides of tubercles (Fig. 2c).

Anterior pole and micropyle: outer chorionic cells diminish somewhat in length towards anterior pole, becoming narrower, with outer chorionic cells reduced to single row (Fig. 3a, b). Tubercles not all separated by uniform gaps (Fig. 3a), many gaps narrower and shallow. Many tubercles close together or almost fused, appearing more rounded and less distinct, especially just posterior to micropyle (Fig. 3b). Anterior ring present but not well formed, usually incomplete (Fig. 3c, d), diameter 35–45 μm, variable, and of very variable width (0–7 μm). Micropylar collar not prominent (Fig. 3a), height 6–10 μm and variable, diameter 20–28 μm and not always circular or continuous (Fig. 3c, d), internal diameter 18–23 μm, wall width 2–6 μm, very variable,

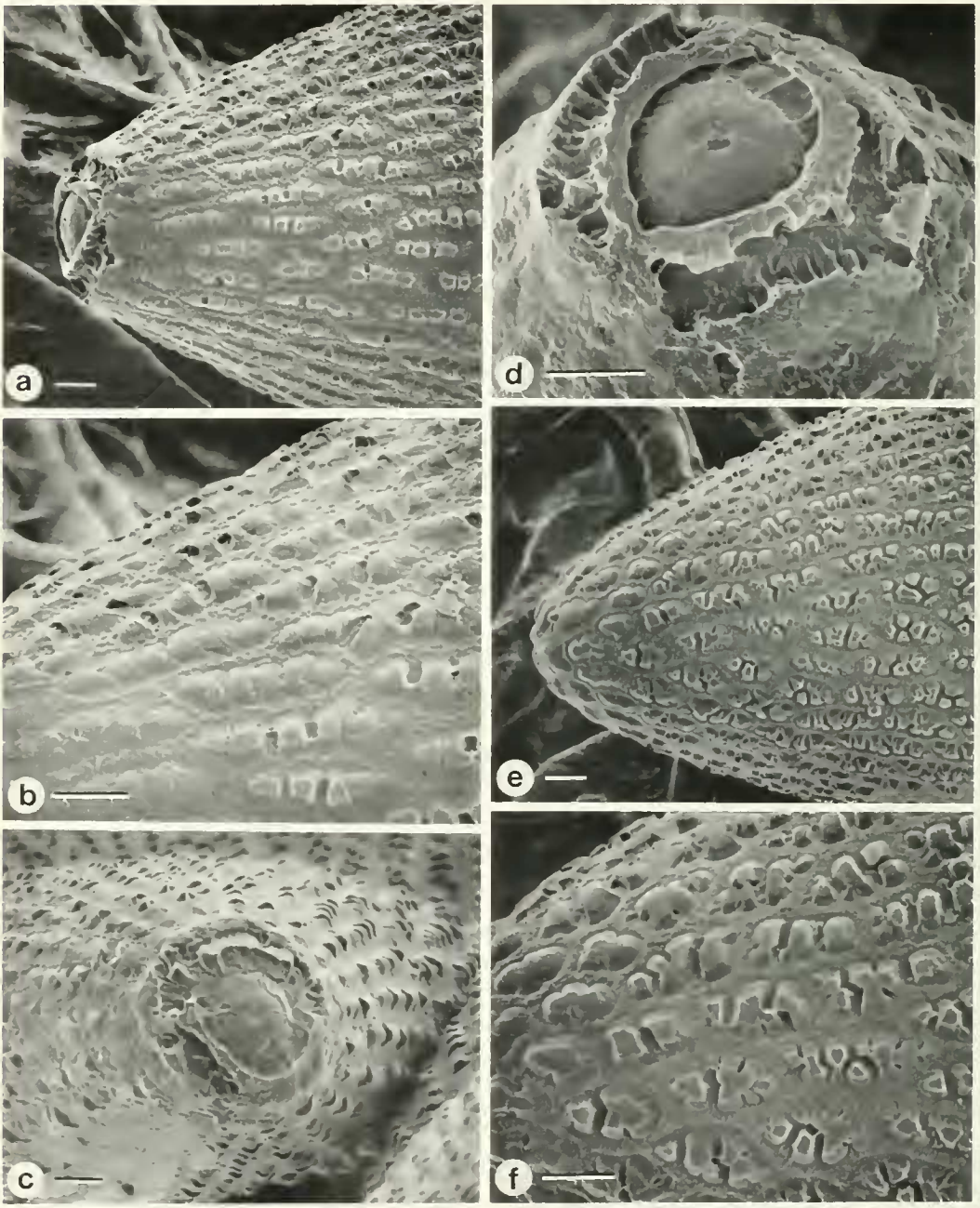


Fig. 3. *Ae. vexans.* (a) Anterior pole and micropylar apparatus, lateral surface; (b) anterior pole, lateral surface, chorionic cell detail; (c) top view, anterior pole and micropylar apparatus; (d) top view, detail of micropylar apparatus; (e) posterior pole, lateral surface; (f) posterior pole, ventral surface, outer chorionic cell detail. Scale = 10 μm.

Table 1. Dimensions of the eggs of two species of *Aedes* (n = 15).

Species	Length (μm)		Width (μm)		L/W Ratio	
	Mean (±SE)	Range	Mean (±SE)	Range	Mean (±SE)	Range
Ae. vexans	650.9 ± 3.3	637.3–665.3	197.6 ± 1.8	193.7–203.9	3.29 ± 0.03	3.14–3.54
Ae. infirmatus	664.4 ± 3.6	637.3–685.7	207.8 ± 1.9	201.4–224.3	3.20 ± 0.03	3.02–3.40

outer margin irregular. Micropylar disc fairly clearly defined, domed (Fig. 3c, d), diameter 10–18 μm, micropyle indistinctly trilobed, diameter ca. 2.6 μm.

Posterior pole: outer chorionic cells become shorter near posterior pole, gaps between outer chorionic tubercles become more irregular (Fig. 3e), some tubercles fused and all fused in cells immediately at and adjacent to pole (Fig. 3e, f).

Aedes (*Ochlerotatus*) *infirmatus* (Figs. 4–6)

Size: dimensions as in Table 1.

Color: satiny black.

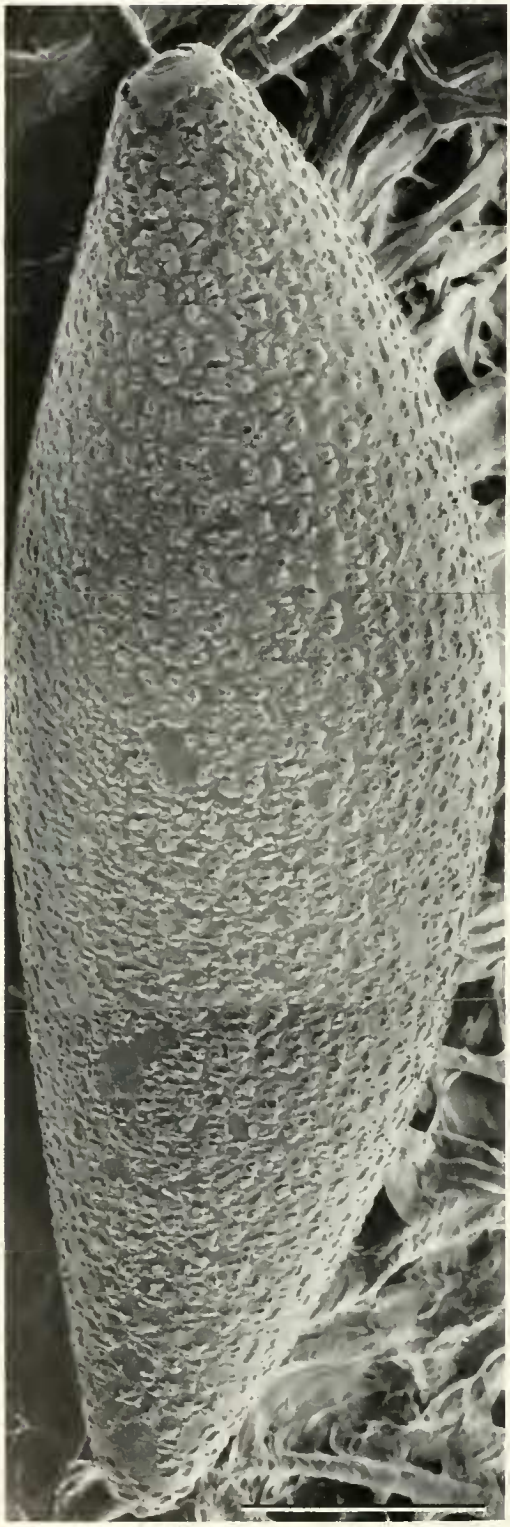
Overall appearance: shape fusiform, somewhat variable, ventral surface more convex than dorsal, widest point just anterior to middle, anterior taper more abrupt than posterior, both anterior and posterior dorsal margins straighter than more curved ventral margins (Fig. 4). Outer chorionic cells appearing irregular in outline, boundaries difficult to distinguish, outer chorionic tubercles clearly visible and many quite large, but not conforming to any easily discernible pattern (Fig. 4). Micropylar collar relatively inconspicuous, conforming to taper of egg.

Chorion, ventral surface: outer chorionic cells somewhat longitudinally elongate, polygonal, but very variable in form, 20–26 μm long, 10–13 μm wide, boundaries quite straight (Fig. 5a), corresponding cell field dimensions ca. 17–25 μm and 9–11 μm. Outer chorionic tubercles 2–7 in number, fewer than on lateral and dorsal surfaces (Table 2), more or less evenly spaced longitudinally in cell, usually but not always touching outer chorionic reticulum on at least one side (Fig. 5a, d). Largest tubercles 6–11 μm long (longest dimension), some as small as 1.2 μm, but very small tubercles fairly uncommon. Form of tubercles complex and irregular, each consisting of a low, smooth base, often with deeply excavated outline (Fig. 5a, d) and a smaller, domed upper portion, which is somewhat less irregular in outline and covered with small rounded bumps (Fig. 5d). Tubercles mostly separated from one another, but occasionally joined by narrow bridges (Fig. 5a). Floors of outer chorionic cell fields smooth, but partly covered with a very thin layer of irregular outline usually extending some distance from field edges, but occasionally forming narrow bridges to tubercles or completely across cell (Fig. 5d). Outer chorionic reticulum low, width 0.9–3.2 μm, consisting of a fine reticulate meshwork with a line of small (ca. 0.3–0.5 μm diameter), rather un-

Table 2. Numbers of outer chorionic tubercles in outer chorionic cells on different egg surfaces of two species of *Aedes* (n = 15).

Species	Dorsal Surface		Lateral Surface		Ventral Surface	
	Mean (±SE)	Range	Mean (±SE)	Range	Mean (±SE)	Range
Ae. vexans	8.7 ± 0.7	6–14	*		10.0 ± 0.5	7–15
Ae. infirmatus	5.5 ± 0.4	3–8	9.6 ± 0.4	7–12	3.7 ± 0.3	2–7

* Not counted.



evenly spaced (0.6–2.2 μm) round or tab-like protuberances almost always offset to one side of reticulum and often attached to adjacent cell floor (Fig. 5d). Meshwork of reticulum often overlying bases of tubercles, particularly at cell corners (Fig. 5d).

Chorion, lateral surface (ventral-dorsal transition): outer chorionic cells not polygonal, outlines much more rounded, somewhat longitudinally elongate, but also with at least one or, more frequently, two extensions in the circumferential direction (Fig. 5b). Cell length (longitudinal) 20–23 μm, width including circumferential extensions 19–30 μm, corresponding cell field dimensions 17–20 μm and 16–27 μm. Outer chorionic tubercles 7–12 in number, more than on ventral or dorsal surfaces (Table 2), large central ones often not touching outer chorionic reticulum, bridges joining adjacent tubercles quite frequent (Fig. 5b). Structure of tubercles, cell fields and outer chorionic reticulum same as on ventral surface.

Chorion, dorsal surface: shape of outer chorionic cells irregular, boundaries rounded (Fig. 5c), cells not as wide in longitudinal direction (12–19 μm) as circumferential (12–31 μm), number of tubercles 3–8, more than on ventral but fewer than on lateral surfaces (Table 2). Outer chorionic tubercles very irregular in shape, almost always touching outer chorionic reticulum on at least part of one side (Fig. 5c), detailed structure same as ventral surface except that nodular texture of top surface less clearly defined (Fig. 5e). Outer chorionic reticulum and cell fields same as on ventral surface (Fig. 5e).

Anterior pole and micropyle: outer chorionic cells become smaller towards anterior pole, numbers of outer chorionic tubercles in each cell fewer, tubercles often partly or almost completely fused (Fig. 6a). Anterior

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Fig. 4. *Ae. infirmatus*. Entire egg, lateral view; dorsal surface at left, anterior end at top. Scale = 100 μm.

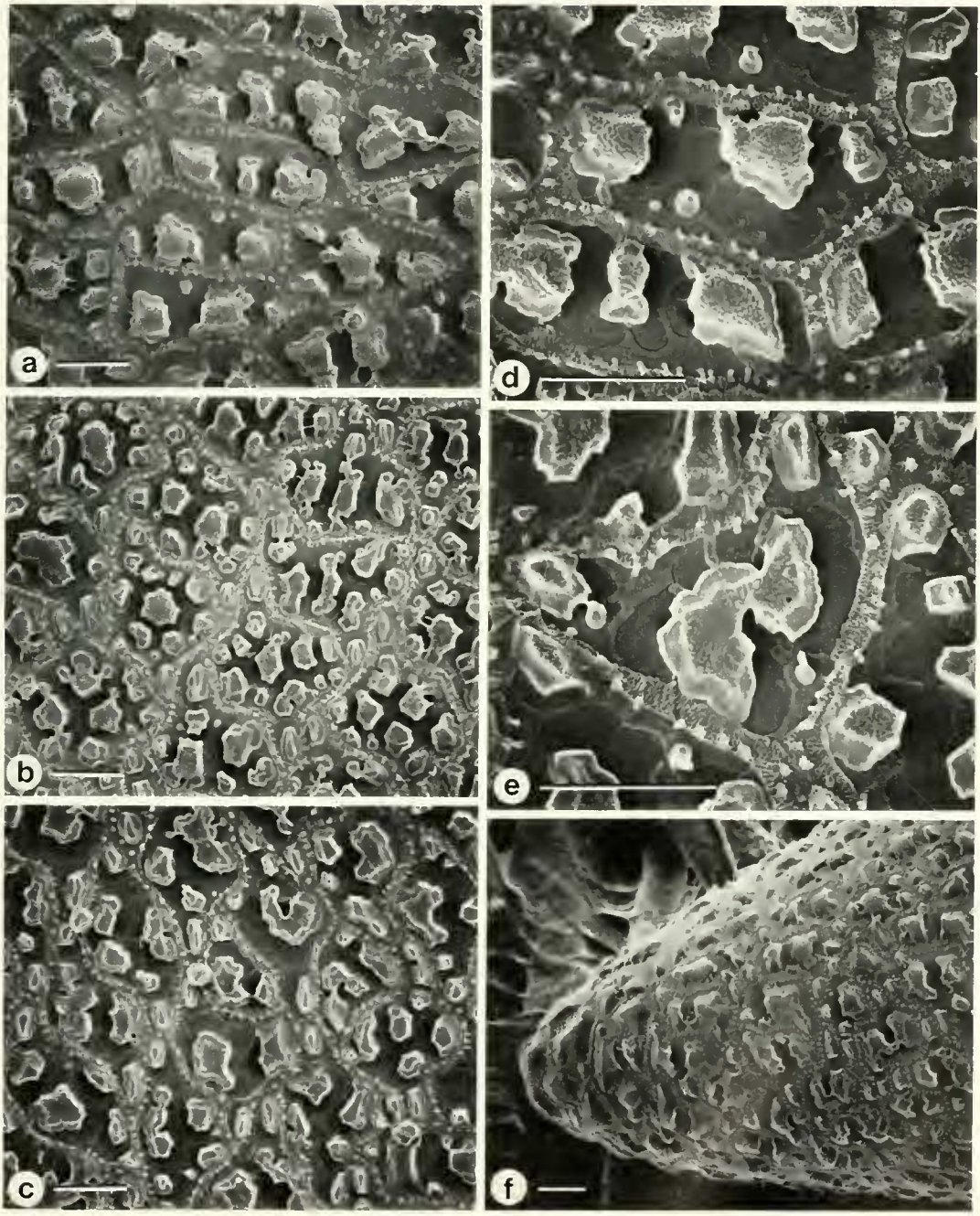

Fig. 5. *Ae. infirmatus.* (a) Outer chorionic cells, ventral surface; (b) outer chorionic cells, lateral surface; (c) outer chorionic cells, dorsal surface; (d) detail of outer chorionic cell and reticulum, ventral surface; (e) detail of outer chorionic cell and reticulum, dorsal surface; (f) posterior pole, lateral surface. Scale = 10 μm.

ring absent. Micropylar collar tapered in conformity with rest of egg and therefore not conspicuous, height 7–10 μm, variable, diameter 26–40 μm, more or less circular but not always continuous (Fig. 6b, c), internal diameter 20–26 μm, wall width 3–9 μm and very variable (Fig. 6c), interior wall appearing as a series of shallow excavations (Fig. 6b, c). Micropylar disc fairly prominent, diameter 17–19 μm, with quite conspicuous (Fig. 6c) central dome (ca. 14 μm in diameter). Micropyle roughly circular, diameter ca. 1.8 μm.

Posterior pole: outer chorionic cells diminish in size towards pole, boundaries irregular (Fig. 5f), outer chorionic cells progressively fewer in number, becoming fused to form very large tubercles, until all tubercles fused in cells crowning posterior pole (Fig. 5f).

Discussion

In the several earlier accounts of *Aedes* eggs (Horsfall and Craig 1956, Craig and Horsfall 1960, Myers 1967, Kalpage and Brust 1968, Horsfall et al. 1970), the principal characters described were color, size, shape, and the inner chorionic pattern, representing the boundaries of the chorionic cells. Keys for identifying eggs of different species could be constructed using these characters (Myers 1967, Kalpage and Brust 1968). However, examination of the inner chorionic pattern relied on phase contrast microscopy following a rather lengthy preparative procedure (Craig 1955) involving removal of the embryo and the outer chorion, then bleaching, washing, dehydrating, clearing and mounting pieces of the inner chorion in balsam. This method has the advantage that it can be carried out with minimal equipment and requires only relatively simple techniques of light microscopy. Its great disadvantage is that it destroys a major part of the intact structure of the egg. The outer chorion is complex (e.g. Figs. 2, 5) and embodies a number of characters of potential taxonomic interest.

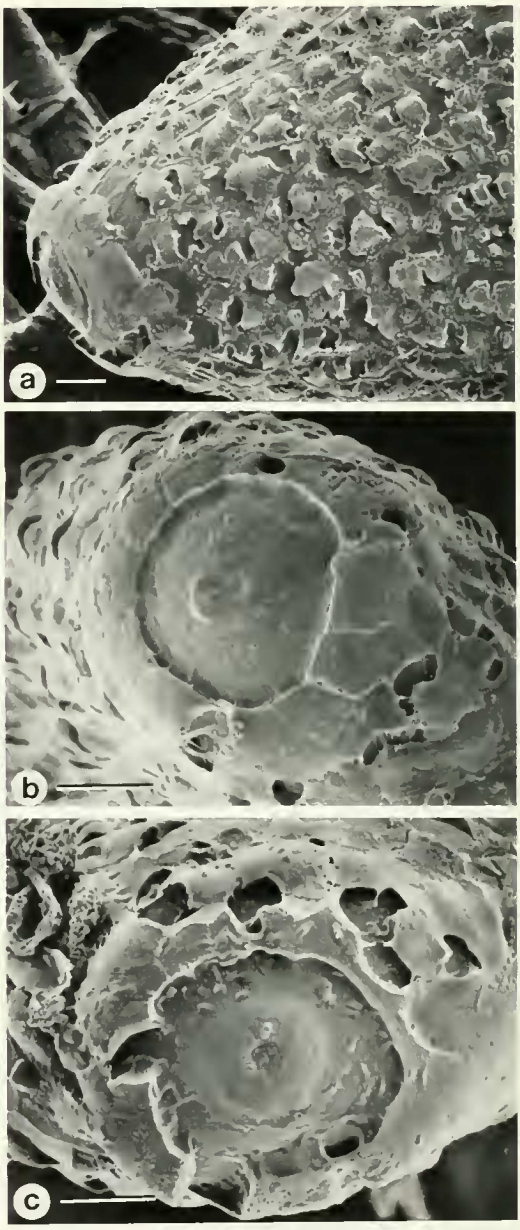

Fig. 6. *Ae. infirmatus.* (a) Anterior pole and micropylar apparatus, lateral surface; (b) top view, detail of micropylar apparatus; (c) variant of micropylar apparatus. Scale = 10 μm.

It is true that eggs handled excessively, as may be unavoidable in field collections, tend to lose at least some of the outer chorion and that outer chorionic details cannot nor-

mally be discerned by light microscopy. However, scanning electron micrographs (e.g. Figs. 1, 4) compared to phase contrast images of the chorionic obviously represent a major improvement towards understanding the intact structure and appearance of these eggs. Existing earlier descriptions are useful, but re-examination is useful in view of the enhancements obtainable from electron microscopy. The preparative work required, at least for *Aedes* eggs, is considerably less than for phase contrast microscopy of the inner chorion (Craig 1955).

In intact eggs, the main areas of new detail revealed in the electron micrograph are in the outer chorionic tubercles particularly and also the chorionic reticulum. In this paper I have not explored the several potential quantitative characters in these structures in any depth. Such an inquiry will only be useful after eggs of many other species, or different geographic populations of single species have been examined and are available on stubs for possible additional and more detailed study. Several characters might be used, such as (i) size distribution of the outer chorionic tubercles, (ii) shape of tubercles and, related to this, (iii) characteristics of their boundaries, (iv) distance separating tubercles, (v) frequency of bridges joining tubercles and, (vi) frequency of distribution in different areas of the cell field. Probably in all of these *Aedes* eggs there are at least some differences between the outer chorionic cells on different surfaces of the egg, as in *Aedes infirmatus* (Fig. 5a, b, c), and it will be necessary to select specific areas of the egg surface for study and measurement.

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